Calcium and Contractility Measurements and Adenoviral Infection

Supplemental Methods
AfCS Research Reports CM0005
Version 1, 04/01/03

Calcium and contractility measurements

Myocytes were isolated and plated in glass bottom 35-mm dishes (MatTek Corporation, Ashland, MA), and cultured in a 2% CO_2 37^{o}C incubator for 18-22 hr. After 18-22 hr, culture medium was removed and myocytes were loaded with 1 μM fura-2 AM (Molecular Probes, Eugene, OR) in Hank's Balanced Salt Solution (HBSS)-BSA (AfCS solution protocol ID PS00000032) for 15 min at 37^{o}C in an environmental chamber without CO_2 under a dark cover to prevent photobleaching. The fura-2 AM-containing medium was then replaced with 2 ml of fresh HBSS-BSA, and the 35 mm dish containing the myocytes was placed on the stage of an inverted microscope (Olympus, IX-70). An electrode was inserted into the 35-mm dish and electrical stimulation was applied through a field stimulator (IonOptix) at 20V, 1 Hz for 4 ms duration. An integrated system (IonOptix Corporation, Milton, MA) with a video-based edge-detection device and dual-excitation (360 nm and 380 nm wavelength) fluorescence photomultiplier was used to monitor the myocyte calcium transients and contractility changes using a Uapo/340 20X lens (Olympus). All the measurements were performed at ambient temperature. Only quiescent, rod-shaped myocytes with clear edges were selected for recording. Soft-edge software (IonOptix) was used to capture changes in cell length and intracellular fluorescence intensity during contraction and relaxation. Basal calcium transients and contractility were measured for 5 min and then 1 μM isoproterenol (ISO) was added (in a 1 ml volume without agitation) and calcium transients and contractility were recorded for 5 min after ligand addition. Each measurement was obtained from one myocyte per 35-mm culture dish, and data from multiple measurements were pooled. Post-acquisition data analysis was performed with IonOptix software.

Adenoviral Infection of Adult Mouse Cardiac Myocytes

Myocytes were infected with adenovirus carrying a β-galactosidase reporter gene (generous gift from Dr. Wally Koch, Duke University). After plating the myocytes, varying titers of adenovirus (multiplicity of infection range: 30-1000) diluted in phosphate buffered saline (PBS) pH 7.3 were added directly to the myocyte culture medium and cultured for 72 hr.

After 24 or 72 hr, myocytes were assayed for β-galactosidase enzyme activity with a colorimetric assay using the β-galactosidase substrate, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal). Myocytes were fixed with 0.1% gluteraldehyde in PBS, pH 7.3 for 10 min at room temperature and washed twice with PBS. Myocytes were then stained for β-galactosidase activity at 30°C for 16 hr using 1 mg/ml X-Gal substrate in PBS with 10 mM Tris base, pH 7.3, 2 mM $MgCl_2$, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$-$3H_2O$, 0.01% sodium deoxycholate,

and 0.02% NP40. After staining, myocytes were washed twice with PBS and photographed under phase microscopy.